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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
09/529,722	04/19/2000	DAVID J SQUIRRELL	124-765	3335

23117 7590 11/18/2003

NIXON & VANDERHYE, PC
1100 N GLEBE ROAD
8TH FLOOR
ARLINGTON, VA 22201-4714

EXAMINER	
STEADMAN, DAVID J	

ART UNIT	PAPER NUMBER
1652	

DATE MAILED: 11/18/2003

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BEFORE THE BOARD OF PATENT APPEALS AND INTERFERENCES

Paper No. 11042003

Application Number: 09/529,722
Filing Date: April 19, 2000
Appellant(s): SQUIRRELL ET AL.

B. J. Sadoff
For Appellant

EXAMINER'S ANSWER

This is in response to the appeal brief filed August 28, 2003.

(1) Real Party in Interest

A statement identifying the real party in interest is contained in the brief.

(2) Related Appeals and Interferences

A statement identifying the related appeals and interferences which will directly affect or be directly affected by or have a bearing on the decision in the pending appeal is contained in the brief.

(3) Status of Claims

The statement of the status of the claims contained in the brief is correct.

(4) Status of Amendments After Final

The appellant's statement of the status of amendments after final rejection contained in the brief is correct.

(5) Summary of Invention

The summary of invention contained in the brief is correct.

(6) Issues

The appellant's statement of the issues in the brief is correct.

(7) Grouping of Claims

Appellant's brief includes a statement that the claims do not stand or fall together and provides reasons as set forth in 37 CFR 1.192(c)(7) and (c)(8). See pages 17-20, 25-27, and 32-34 of the Appeal Brief for those reasons wherein appellant asserts the claims do not stand or fall together. Appellants' statement in the Appeal Brief that certain claims do not stand or fall together is not agreed with and appellants' arguments are addressed in the "Response to Argument" section.

(8) Claims Appealed

The copy of the appealed claims contained in the Appendix to the brief is correct.

(9) Prior Art of Record

Backman et al. EP 0 373 962 (June 1990)

Liang et al. (1989) *Gene* 80:21-28

Kajiyama et al. (1993) *Biochemistry* 32:13795-13799

Kajiyama et al. US Patent 5,229,285* (July 1993)

Belinga et al. (1995) *J Chromat A* 695:33-40

Wood et al. WO 99/14336* (March 1999)

Lowe et al. WO 95/25798 (September 1995)

Squirrell et al. WO 96/22376* (July 1996)

Gilles et al. (1986) *Proc Natl Acad Sci, USA* 83:5798-5802

* indicates reference cited by appellants

(10) Grounds of Rejection

The following ground(s) of rejection are applicable to the appealed claims:

Written Description Rejection Under 35 USC § 112, First Paragraph

The following is a quotation of the first paragraph of 35 U.S.C. 112:

> The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same and shall set forth the best mode contemplated by the inventor of carrying out his invention.

Claims 67-106 are rejected under 35 U.S.C. 112, first paragraph, as failing to comply with the written description requirement. The claim(s) contains subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventor(s), at the time the application was filed, had possession of the claimed invention.

Claims 67-71, 73-79, and 91-94 are drawn to methods for producing a genus of polypeptide products which are substantially free of a genus of mutant undesired protein that hinders the use of the polypeptide product and has activity essential for cell survival as encompassed by the claims. Claims 83, 85-90, 98, and 100-105 are drawn to a recombinant cell transformed to express a first polynucleotide encoding a genus of desired polypeptides and further transformed to express a genus of mutant undesirable contaminating proteins that hinder the use of the polypeptide product and have activity essential for cell survival and methods for producing said recombinant cell as encompassed by the claims. Claims 72, 80-82, 84, 95-97, 99, and 106 limit the genus of polypeptide products to a genus of luciferases and limit the genus of undesired proteins to a genus of adenylate kinases.

The claims are rejected because the structures of the genera of polypeptide products, desired polypeptides, undesired proteins, luciferases (optionally being thermostable and optionally being thermostable at 37 degrees Celsius), and adenylate kinases (optionally being thermosensitive at 37 degrees and including mutations at positions 87 and 107 of *E. coli* adenylate kinase) have not been adequately described in the specification. For claims drawn to a genus, MPEP § 2163 states the written

description requirement for a claimed genus may be satisfied through sufficient description of a representative number of species by actual reduction to practice, reduction to drawings, or by disclosure of relevant, identifying characteristics, i.e., structure or other physical and/or chemical properties, by functional characteristics coupled with a known or disclosed correlation between function and structure, or by a combination of such identifying characteristics, sufficient to show the appellant was in possession of the claimed genus. See *Eli Lilly*, 119 F.3d at 1568, 43 USPQ2d at 1406. MPEP § 2163 states that a representative number of species means that the species which are adequately described are representative of the entire genus. Thus, when there is substantial variation within the genus, one must describe a sufficient variety of species to reflect the variation within the genus. In this case the specification refers only to four representative species of the recited genus of polypeptide products, desired polypeptides, and luciferases (optionally being thermostable and optionally being thermostable at 37 degrees Celsius) as follows: "a thermostable luciferase... ...as described in European Patent Application No. 92 1 10808.0 or WO 95/25798" (page 8, lines 19-21 of the specification). It is noted that European Patent Application No. 92 1 10808.0 is the foreign priority document to which the instant application claims foreign priority. It is further noted that WO 95/25798, i.e., the reference of Lowe et al., describes only the following luciferase mutants: *Photinus pyralis* luciferase with mutation at positions 215 and 354 and *Luciola* luciferase with mutation at positions 217 and 356. Also, the specification discloses only two representative species of the recited genus of undesired proteins and adenylate kinases (optionally being thermosensitive at 37 degrees and including mutations at positions 87 and 107 of *E. coli* adenylate kinase): "mutation at position 87 in [the sequence of *E. coli* adenylate kinase] and/or position 107 in the sequence, produces a mutant form of adenylate kinase enzyme which is labile at low temperatures" (page 5, lines 11-13). The specification fails to describe any additional representative species of the recited genus or even suggest that others were known in the art. In the instant case, the recited genera of polypeptide products, desired polypeptides, and undesired proteins encompasses a vast number of species having widely variant structures and functions. Also, the recited genera of luciferases (optionally being thermostable and optionally being thermostable at 37 degrees Celsius) and adenylate kinases (optionally being thermosensitive at 37 degrees), encompasses species having widely

variant structures – including luciferases and adenylate kinases from any source that have yet to be isolated. As such, the representative species as referred to or disclosed in the specification are insufficient to be representative of the attributes and features of *all* species encompassed by the recited genera. Furthermore, with the exception of claims 82 and 97, it is noted that the claims recite only functional features of the genera of polypeptide products, desired polypeptides, undesired proteins, luciferases, and adenylate kinases – there are no structural features of the recited genera provided in the claims. The CAFC in *UC California v. Eli Lilly*, (43 USPQ2d 1398) stated that: "In claims to genetic material, however a generic statement such as "vertebrate insulin cDNA" or "mammalian insulin cDNA", without more, is not an adequate written description of the genus because it does not distinguish the claimed genus from others, except by function. It does not specifically define any of the genes that fall within its definition. It does not define any structural features commonly possessed by members of the genus that distinguish them from others. One skilled in the art therefore cannot, as one can do with a fully described genus, visualize or recognize the identity of the members of the genus". Similarly with the recited genera of polypeptide products, desired polypeptides, and undesired proteins, luciferases, and adenylate kinases, the functional definition of the genera does not provide any structural information commonly possessed by members of the genera which distinguish the protein species within the genus from other proteins such that one can visualize or recognize the identity of the members of the genus. Thus, given the lack of description of a representative number of polypeptide products, desired polypeptides, undesired proteins, luciferases, and adenylate kinases, the specification fails to sufficiently describe the claimed invention in such full, clear, concise, and exact terms that a skilled artisan would recognize that appellant was in possession of the claimed invention.

Scope of Enablement Rejection Under 35 USC § 112, First Paragraph

Claims 67-106 are rejected under 35 U.S.C. 112, first paragraph, because the specification, while being enabling for: 1) a method for producing thermostable *P. pyralis* luciferase with mutation at position 215 or 354 or a thermostable *Luciola* luciferase with mutation at position 217 or 356 that is substantially free of adenylate kinase activity by transforming a host cell with a nucleic acid encoding said

thermostable *P. pyralis* or *Luciola* luciferase and co-transforming a host cell with a nucleic acid encoding *E. coli* adenylate kinase activity with amino acid substitution at position 87 or 107 and removing adenylate kinase activity by heating the host cell or host cell extract at a temperature sufficient to inactivate the mutant adenylate kinase, while luciferase maintains activity; 2) a recombinant host cell transformed to express a thermostable *P. pyralis* luciferase with mutation at position 215 or 354 or a thermostable *Luciola* luciferase with mutation at position 217 or 356 and an *E. coli* adenylate kinase mutant with amino acid substitution at position 87 or 107; and 3) a method of making said recombinant host cell, does not reasonably provide enablement for: 1) a method of producing any polypeptide products, desired proteins, or luciferases (optionally being thermostable and optionally being thermostable at 37 degrees Celsius) substantially free of any undesired proteins and adenylate kinases (optionally being thermosensitive at 37 degrees and including mutations at positions 87 and 107 of *E. coli* adenylate kinase) that is required for cell viability and interferes with the protein product by treating the expressed polypeptide product to any conditions or any conditions or conditions of temperature or pH at which the undesired protein or adenylate kinase (optionally being thermosensitive at 37 degrees and including mutations at positions 87 and 107 of *E. coli* adenylate kinase) is denatured/inactivated; 2) all recombinant host cells expressing all nucleic acids encoding any polypeptide products, desired polypeptides, or luciferases (optionally being thermostable and optionally being thermostable at 37 degrees Celsius) and any undesired proteins or adenylate kinases (optionally being thermosensitive at 37 degrees and including mutations at positions 87 and 107 of *E. coli* adenylate kinase), wherein the undesired polypeptide has activity that is essential for cell survival in a mutant form such that the desired polypeptide remains unaffected at any conditions or temperature conditions at which an undesired protein is inactivated/denatured; and 3) a method for making said recombinant host cell.

The specification does not enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make the invention commensurate in scope with these claims. It is the examiner's position that undue experimentation would be required for a skilled artisan to make and/or use the entire scope of the claimed invention. Factors to be considered in determining whether undue experimentation is required, are summarized in *In re Wands* (858 F.2d 731, 8 USPQ 2nd 1400 (Fed. Cir,

1988)) as follows: (1) the quantity of experimentation necessary, (2) the amount of direction or guidance presented, (3) the

presence or absence of working examples, (4) the nature of the invention, (5) the state of the prior art, (6) the relative skill of those in the art, (7) the predictability or unpredictability of the art, and (8) the breadth of the claims). The Factors most relevant to the instant rejection are addressed below.

- The claims are overly broad in scope: Regarding claims 67-82, 91-97, and 106, these claims are so broad as to encompass a method of producing any polypeptide products, desired polypeptides, and luciferases (optionally being thermostable and optionally being thermostable at 37 degrees Celsius) that is substantially free of any undesired protein or adenylate kinase (optionally being thermosensitive at 37 degrees and including mutations at positions 87 and 107 of *E. coli* adenylate kinase) that is required for cell viability and interferes with the protein product by treating the expressed polypeptide products, desired polypeptides, or luciferases (optionally being thermostable and optionally being thermostable at 37 degrees Celsius) to any conditions or any conditions of temperature or pH at which the undesired protein is denatured/inactivated. Regarding claims 83-90 and 98-105, these claims are so broad as to encompass all recombinant host cells expressing all nucleic acids encoding any polypeptide products, desired polypeptides, or luciferases (optionally being thermostable and optionally being thermostable at 37 degrees Celsius) and an undesired polypeptide or adenylate kinase (optionally being thermosensitive at 37 degrees and including mutations at positions 87 and 107 of *E. coli* adenylate kinase), wherein the undesired polypeptide or adenylate kinase (optionally being thermosensitive at 37 degrees and including mutations at positions 87 and 107 of *E. coli* adenylate kinase) has activity that is essential for cell survival in a mutant form such that the desired polypeptide remains unaffected at any conditions or temperature conditions at which an undesired protein or adenylate kinase (optionally being thermosensitive at 37 degrees and including mutations at positions 87 and 107 of *E. coli* adenylate kinase) is inactivated/denatured and a method for making said recombinant host cell. The scope of the claims is not commensurate with the enablement provided by the disclosure and the prior art with regard to the large number of protein products, desired polypeptides, undesired proteins, luciferases, and adenylate kinases. As previously stated, the enablement provided by the specification is limited to: 1) a method for producing

thermostable *P. pyralis* luciferase with mutation at position 215 or 354 or a thermostable *Luciola* luciferase with mutation at position 217 or 356 that is substantially free of adenylate kinase activity by transforming a host cell with a nucleic acid encoding said thermostable *P. pyralis* or *Luciola* luciferase and co-transforming a host cell with a nucleic acid encoding *E. coli* adenylate kinase activity with amino acid substitution at position 87 or 107 and removing adenylate kinase activity by heating the host cell or host cell extract at a temperature sufficient to inactivate the mutant adenylate kinase, while lucifierase maintains activity; 2) a recombinant host cell transformed to express a thermostable *P. pyralis* luciferase with mutation at position 215 or 354 or a thermostable *Luciola* luciferase with mutation at position 217 or 356 and an *E. coli* adenylate kinase mutant with amino acid substitution at position 87 or 107; and 3) a method of making said recombinant host cell.

- The lack of guidance and working examples: The specification fails to provide guidance that would enable a skilled artisan to make and/or use the entire scope of claimed recombinant cells and methods. The specification refers to prior art references teaching only the following working examples of polypeptide products, desired polypeptides, or luciferases (optionally being thermostable and optionally being thermostable at 37 degrees Celsius) as encompassed by the claims: thermostable *P. pyralis* luciferase with mutation at position 215 or 354 or a thermostable *Luciola* luciferase with mutation at position 217 or 356. Furthermore, the specification discloses only the following working examples of undesired polypeptides or adenylate kinases (optionally being thermosensitive at 37 degrees and including mutations at positions 87 and 107 of *E. coli* adenylate kinase) as encompassed by the claims: an *E. coli* adenylate kinase mutant with amino acid substitution at position 87 or 107 and the prior art indicates that the mutant adenylate kinase is irreversibly inactivated at 40 degrees Celsius (Gilles et al., page 5798, left column, bottom). Specifically regarding claims reciting luciferases (optionally being thermostable and optionally being thermostable at 37 degrees Celsius) and adenylate kinase (optionally being thermosensitive at 37 degrees and including mutation at position 87 or 107 of *E. coli* adenylate kinase), it is noted that the specification provides no further guidance as to luciferase mutants or adenylate kinase mutants and the claims are not so limited to those thermostable luciferases/thermolabile adenylate kinases that are known in the art. Moreover, it is noted that some claims are not limited to any

particular conditions under which the protein product/desired polypeptide/luciferase maintains activity and the denaturation/inactivation of the undesired protein/adenylate kinase occurs. For example, claim 67 part (e) and claim 79 recite any conditions or pH conditions that inactivate the undesired polypeptide, while the protein product/desired polypeptide maintains activity. With the exception of temperature conditions relating to the thermostability of luciferase and thermolability of adenylate kinase, the specification fails to provide guidance regarding desired proteins/protein products/luciferases that are stable under any other conditions or pH conditions that an undesired protein/adenylate kinase is inactivated/denatured. No other guidance or working examples are provided in the specification, and even in view of the prior art, a skilled artisan would not be able to make the FULL scope of claimed recombinant cells and methods.

- The high degree of unpredictability of the art: The ability of any protein product/desired protein/luciferase to maintain activity at any conditions/temperature conditions/pH conditions at which any undesired protein/adenylate kinase is denatured is highly unpredictable. Similarly, the inactivation/denaturation of any undesired protein/adenylate kinase under any conditions or pH conditions at which any undesired protein is denatured is highly unpredictable. The working examples as taught by appellant are insufficient to provide a skilled artisan with a reasonable expectation of success for making the broad scope of the claimed invention. While methods of making mutant proteins and mutant encoding nucleic acid sequences are known, the ability to predict which mutations of any protein product/desired protein/luciferase will result in an increased tolerance to any conditions, temperature, or pH or alternatively, the ability to predict which mutations of any undesired protein/adenylate kinase will result in a decreased tolerance to any conditions/temperature/pH is outside the ability of a skilled artisan, particularly in view of the lack of guidance and working examples provided in the specification and the prior art. Since the amino acid sequence of a protein determines its structural and functional properties, predictability of which changes can be tolerated in a protein's amino acid sequence and obtain the desired activity requires a knowledge of and guidance with regard to which amino acids in the protein's sequence, if any, are tolerant of modification and which are conserved (i.e. expectedly intolerant to modification), and detailed knowledge of the ways in which the proteins' structure relates to its function. The high degree of unpredictability is evidenced by Gilles et al. who teach, "a single amino acid

substitution may be responsible for changes in protein structure and catalytic activity" (page 5801, left column). In this case, the necessary guidance has not been presented. Even if one were to isolate mutants of any protein product/desired protein/luciferase with an increased tolerance to any conditions, temperature, or pH and mutant undesired proteins/adenylate kinases with decreased tolerance to any conditions/temperature or pH, respectively, it is highly unpredictable whether the combination of proteins will be useful in the claimed methods.

- The amount of experimentation: While recombinant and mutagenesis techniques are known, it is not routine in the art to screen for all polypeptide products, desired polypeptides, luciferases (optionally being thermostable and optionally being thermostable at 37 degrees Celsius), undesired polypeptides and adenylate kinases (optionally being thermosensitive at 37 degrees and optionally including mutations at positions 87 and 107 of *E. coli* adenylate kinase) with an increased or decreased tolerance to any conditions or conditions of temperature or pH, as encompassed by the instant claims. In view of the broad scope of the claims, the lack of guidance and working examples, and the high degree of unpredictability, the amount of experimentation required to make the broad scope of claimed recombinant cells and methods would clearly constitute undue experimentation.

Thus, appellants have not provided sufficient guidance to enable one of ordinary skill in the art to make and use the claimed invention in a manner reasonably correlated with the scope of the claims. The scope of the claims must bear a reasonable correlation with the scope of enablement *In re Fisher*, 166 USPQ 19 24 (CCPA 1970)). Without sufficient guidance, determination of having the desired biological characteristics is unpredictable and the experimentation left to those skilled in the art is unnecessarily, and improperly, extensive and undue. See *In re wands* 858 F.2d 731, 8 USPQ2nd 1400 (Fed. Cir, 1988).

Obviousness Rejection Under 35 USC § 103(a)

The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art

to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

Claims 67-78 and 80-106 are rejected under 35 U.S.C. 103(a) as being unpatentable over Backman et al. (EP 373962) in view of Kajiyama et al. (*Biochemistry* 32:13795-13799), Gilles et al. (*Proc Natl Acad Sci, USA* 83:5798-5802), and Belinga et al. (*J Chromat A* 695:33-40). Claims 67-82, 91-97, and 106 are drawn to methods for producing polypeptide products free of an undesired protein or luciferase free of adenylate kinase as encompassed by the claims. Claims 83-87 and 98-102 are drawn to recombinant cells expressing a desired protein and an undesired protein or luciferase and adenylate kinase as encompassed by the claims and claims 88-90 and 103-105 are drawn to methods for producing said recombinant cell as encompassed by the claims.

Backman et al. teach the following method: "a method for obtaining a thermostable enzyme essentially free from unwanted contaminants, characterized in comprising the steps of: (a) providing a mesophilic host cell engineered to express a gene encoding a heterologous thermostable enzyme; (b) culturing said mesophilic host cell to produce said thermostable enzyme in a mixture comprising unwanted contaminants; and (c) purifying said thermostable enzyme said purification comprising at least one step in which a mixture comprising said unwanted contaminants is heated to a temperature sufficient to inactivate said unwanted contaminants but not sufficient to inactivate said thermostable enzyme" (column 2, lines 22-37). Backman et al. characterize the contaminating substances particularly as those that "interfere with the intended use of the enzyme" (column 2, lines 38-43). Backman et al. teach, "[m]esophilic host cells are cells which can be engineered to produce the desired thermophilic enzyme and whose proteins generally are denatured at a temperature that does not denature the desired thermophilic enzyme" (column 2, lines 45-49). Backman et al. do not teach expressing an undesired protein required for cell survival in a mutant form such that the mutant protein is inactivated under a temperature of 37 degrees Celsius wherein the desired thermostable protein remains active.

Kajiyama et al. teach vectors encoding a mutant thermostable *Luciola cruciata* luciferase (page 13796, left column, bottom to right column top and Table 1). Sequencing the plasmid indicated the mutation resulting in the thermostable luciferase is a substitution of threonine with isoleucine at amino

acid position 217 (page 13796, right column, top and Table 1). Kajiyama et al. teach expressing their vector encoding a thermostable luciferase in an *E. coli* host cell (page 13795, right column, middle) and a comparison of thermostability of the wild-type and the mutants indicated that the wild-type *L. cruciata* luciferase in cell extracts was inactivated by incubation at 50 degrees Celsius for 40 minutes, while the thermostable luciferase with in cell extracts maintained over 30 % enzymatic activity (page 13796, left column, bottom and Figure 2). Kajiyama et al. teach substitution at amino acid position 217 of *L. cruciata* luciferase with Leu resulted in a mutant thermostable luciferase that when incubated at 50 degrees Celsius for 60 minutes was able to maintain 50% activity.

Gilles et al. teach thermosensitive *E. coli* mutants comprising a mutation in the endogenous *adk* gene encoding adenylate kinase (page 5798, left column, middle). Characterization of the mutant adenylate kinase protein revealed the presence of a substitution of serine for proline at amino acid position 87 (page 5798, right column, top). Gilles et al. teach thermosensitive adenylate kinase is irreversibly inactivated at 40 degrees Celsius due to proteolysis subsequent to thermal denaturation (page 5798, left column, bottom) and that this increased susceptibility to proteolysis is enhanced by increased temperature (page 5801, right column, middle). Gilles et al. teach that an analysis of purified wild-type and mutant adenylate kinase indicated that the mutant loses significant enzyme activity as the reaction temperature is increased from 27 degrees Celsius to 40 degrees Celsius (page 5802, Table 4) and that these changes in kinetic parameters of the mutant are most likely due to destabilization of the protein molecule which is enhanced by increased temperature (page 5802, left column, top).

Belinga et al. provide motivation for removing adenylate kinase activity in a luciferase preparation. Belinga et al. teach the presence of adenylate kinase interferes with luciferase bioluminescence assays and it is necessary to remove adenylate kinase during purification of luciferase (page 33, left column, middle). Belinga et al. teach that even after purification of luciferase by their method there remained residual adenylate kinase activity (page 37, left column).

Therefore, it would have been obvious to one of ordinary skill in the art at the time of the invention to combine the teachings of Backman et al., Belinga et al., Kajiyama et al., and Gilles et al. to transform the mutant *E. coli* of Gilles et al. expressing an endogenous thermosensitive adenylate kinase or an *E.*

coli host with a disrupted *adk* gene transformed with a plasmid expressing the thermosensitive mutant adenylate kinase of Gilles et al. with the vector encoding the mutant thermostable luciferase of Kajiyama et al., express the mutant thermostable luciferase and heat the resulting cell or cell extract at a temperature sufficient to reduce adenylate kinase activity by denaturation and optionally further purify the expressed thermostable luciferase. One would have been motivated to transform the thermosensitive mutant *E. coli* of Gilles or an *E. coli* host with a disrupted *adk* gene transformed with a plasmid expressing the thermosensitive mutant adenylate kinase of Gilles et al. with the vector encoding the thermostable luciferase of Kajiyama et al., express the encoded luciferase mutant and heat the resulting cell or cell extract at a temperature sufficient to reduce or eliminate adenylate kinase activity because of the teachings of Belinga et al. who teach that is necessary to remove the contaminating adenylate kinase from luciferase. One would have a reasonable expectation of success for transforming the mutant *E. coli* of Gilles et al. or an *E. coli* host with a disrupted *adk* gene transformed with a plasmid expressing the thermosensitive mutant adenylate kinase of Gilles et al. with the vector encoding the mutant thermostable luciferase of Kajiyama et al., expressing the mutant luciferase and heating the resulting cell or cell extract at a temperature sufficient to reduce or eliminate adenylate kinase activity because of the results of Backman et al., Kajiyama et al., and Gilles et al. Therefore, claims 67-82, 91-97, and 106, drawn to methods for producing polypeptide products free of an undesired protein or luciferase free of adenylate kinase as encompassed by the claims, claims 83-87 and 98-102, drawn to recombinant cells expressing a desired protein and an undesired protein or luciferase and adenylate kinase as encompassed by the claims and claims 88-90 and 103-105, drawn to methods for producing said recombinant cell as encompassed by the claims, would have been obvious to one of ordinary skill in the art at the time of the invention.

(11) Response to Argument

Written Description Rejection Under 35 USC § 112, First Paragraph

Appellants' arguments addressing the instant rejection begin at page 10 of the Appeal Brief. Beginning at the top of page 11 and continuing through to the bottom of page 12 of the Brief, appellants

provide "clarification" of the examiner's statement of the claimed invention. Appellants' "clarification" is acknowledged. It is noted that the significant number of claims necessitated summarization by the examiner in the written description rejection under 35 USC § 112, first paragraph. This summary is no way an attempt to mis-construe the claims or the claimed invention.

Beginning at the top of page 13 of the Brief, appellants argue the claimed invention is applicable to a wide range of protein products and nucleic acids encoding undesired proteins. Appellants present, for the first time, the claimed invention is applicable to the following: 1) removal of protease activity for long-term storage of a desired protein; 2) removal of DNase activity from a DNA processing enzyme; and 3) removal of ATPase activity from a luciferase.

Appellants' statements are acknowledged. The applicability of the claimed invention to other proteins in a general sense is not at issue. The issue is whether the specification provides adequate written description of a representative number of species of the recited genera of proteins or encoding nucleic acids. While the claimed invention may be applicable to proteins other than luciferase and adenylate kinase, the specification fails to describe a *representative number of species* of the recited genera of proteins or encoding nucleic acids encompassed by the claims. It should be noted that while appellants recite herein examples of pairs of desired proteins/undesired proteins, the specification does not teach species of any of these protein pairs which differ in stability with respect to temperature, pH or any other conditions such that they could be used in the claimed methods.

Beginning at the middle of page 14 of the Brief, appellants argue the claimed invention requires a combination of elements that were well-known and/or identified or obtainable by routine experimentation at the time of the invention. Appellants argue that thermostable luciferases were known in the art at the time of the invention as Kajiyama et al. (US Patent 5,229,285) disclose a thermostable luciferase and Lowe et al. teach that the thermal and pH stability of the luciferase of Kajiyama et al. (US Patent 5,229,285) is increased. Appellants argue Lowe et al. further teach methods for isolating temperature and/or pH sensitive mutant luciferases and methods of using same. Appellants argue that additional thermostable luciferase mutants are disclosed in Squirrell et al. Appellants argue the teachings of Lowe et al. and Squirrell et al. are examples of the advanced level of skill in the art at the time of the invention and

should not be required to describe that which was well known in the art. Appellants argue Belinga et al. describe standard purification techniques and that identification and use of a variety of specific species having the desired characteristics of the present claims would have been routine to a skilled artisan. Beyond identification of natural sources, appellants argue Kajiyama et al. (US Patent 5,229,285) teach mutagenesis of luciferase-encoding genes can be affected according to methods known in the art. Appellants' arguments are not found persuasive.

While the structures of those thermostable luciferases disclosed in the cited prior art (Kajiyama et al. (US Patent 5,229,285), Lowe et al., and Squirrell et al.) were known in the art at the time of the invention, it is noted that the instant claims are not so limited to the thermostable luciferases referred to in the specification at page 8, line 21 ("[a] luciferase plasmid, preferably a thermostable luciferase plasmid, for example as described in European Patent Application No. 92110808.0 or W095/25798") or those described in the prior art of record. Instead, the claims encompass a vast number of species that are widely variant in both structure and function. Most of the claims are not limited in the scope of protein product/desired protein at all and instead encompass any protein under the sun. Clearly all proteins are not similar to the species of thermostable luciferases described in the specification by either structure or function. Even for those claims that are limited in scope to luciferases as protein products/desired proteins (i.e., claims 72, 80-82, 84, 95-97, 99, and 106), the species referred to by the disclosure are not representative of the structures of other thermostable luciferases because the species encompassed by the genus are widely variant in structure encompassing luciferases from any source including those yet to be isolated. Moreover, there is no way that a mutation within one luciferase that results in an increased thermostability will have a similar effect when applied to other luciferase sequences. The structures of the thermostable luciferases as referred to in the specification and disclosed in the prior art fail to represent the structures and/or functions of the entire genus of recited polypeptide products or luciferases as recited in the claims. As such, the claims fail to meet the written description requirements of 35 USC § 112, first paragraph.

Beginning at the middle of page 17 of the Brief, appellants argue Liang et al. teach cloning of a gene encoding a thermolabile mutant adenylate kinase from *E. coli.* Appellants argue that methods and

materials required to produce a protein that hinders the use of a polypeptide product, which also has an essential activity for host survival, such as adenylate kinase, were well described in the art at the time of the invention. Appellants argue that a description of additional species falling within the claims should not be required. Appellants' arguments are not found persuasive.

While the structures of nucleic acids encoding thermolabile adenylate kinases disclosed in the cited prior art (Gilles et al. and Liang et al.) were known in the art at the time of the invention, it is noted that the instant claims are not so limited to the thermolabile adenylate kinases disclosed in the specification at page 5, lines 1-13 ("[I]t has been found that mutation at position 87 [of *E. coli* adenylate kinase] in that sequence and/or position 107 in the sequence, produces a mutant form of adenylate kinase enzyme which is labile at low temperatures") or those described in the prior art of record. Instead, the claims encompass a vast number of species that are widely variant in both structure and function. As stated above, most of the claims are not limited in the scope of protein product/desired protein at all and instead encompass any protein. Clearly all proteins are not similar to the species of thermolabile adenylate kinases described in the specification by either structure or function. Even for those claims that are limited in scope to adenylate kinases as undesired proteins, the species disclosed in the specification are not representative of the structures of other thermolabile adenylate kinases because the species encompassed by the genus are widely variant in structure encompassing adenylate kinases from any source including those yet to be isolated. Moreover, there is no way that a mutation within one adenylate kinase that results in decreased thermolability will have a similar effect when applied to other adenylate kinase sequences. The structures of the thermolabile adenylate kinases as referred to in the specification and disclosed in the prior art fail to represent the structures and/or functions of the entire genus of recited polypeptide products or luciferases as recited in the claims. As such, the claims fail to meet the written description requirements of 35 USC § 112, first paragraph.

Separate Patentability

Appellants' arguments addressing the separate patentability of the claims rejected for a lack of written description begin at the bottom of page 17 of the Brief. Appellants argue the following claim sets

are separately patentable: claims 67-71; claims 72, 80, 84, 95 and 98-102; claims 81 and 96; claims 82 and 97; claims 73-79, 83, 85-94 and 103-105; and claim 106 are submitted to be each separately patentable one to the other.

Addressing the group of claims 67-71, appellants argue a more detailed description should not be required for identifying mutant forms of the polypeptide product and undesired protein as means are known in the art for identification and use of said mutant forms. Appellants' argument is not found persuasive.

Appellants' arguments appear to address the scope of enablement rejection and not the instant written description rejection. The instant rejection is based on a failure to describe a representative number of species of the recited mutant polypeptides – not whether further description is required to make and use the mutant proteins. Even assuming *arguendo* the specification fully describes the genus of mutant proteins as recited in the claims, the issue of written description in regards to these mutant polypeptide products and undesired proteins has been fully addressed above. For these reasons, it is submitted that the specification does not adequately describe the genus of recited mutant polypeptide products and undesired proteins.

Addressing the group of claims 72, 80, 84, 95 and 90-102, appellants argue luciferase and adenylate kinase as recited in the claims are described in the specification and are known to those of skill in the art such that further description should not be required. Appellants' argument is not found persuasive.

It should be noted that, while claims 72, 80-82, 84, 95-97, 99, and 106 specifically recite luciferase and adenylate kinase, claims 90-94, 98, and 100-102 do not recite these limitations as asserted by appellants. Addressing those claims that do recite luciferase and adenylate kinase, it is noted that the instant claims are not so limited to the thermostable luciferases/thermolabile adenylate kinases referred to or disclosed in the specification and described in the prior art of record. Instead, claims 90-94, 98, and 100-102 encompass a vast number of species that are widely variant in structure and function. Addressing claims 72, 80-82, 84, 95-97, 99, and 106, the structures of the thermostable luciferases/thermolabile adenylate kinases as referred to in the specification and disclosed in the prior art

fail to represent the structures and/or functions of the entire genus of recited polypeptide products, desired proteins, luciferases, undesired proteins, and adenylate kinases as recited in the claims. The recited genera encompasses widely variant structures of adenylate kinase and luciferase. The species encompassed by the genera include any adenylate kinases and luciferases having any mutation(s), including those adenylate kinases and luciferases that have yet to be isolated. As such, the claims fail to meet the written description requirements of 35 USC § 112, first paragraph.

Addressing the group of claims 81 and 96, appellants argue the claims recite adenylate kinase which is thermolabile at a temperature of 37 degrees Celsius, which is disclosed in the specification and Gilles et al. as acknowledged by the examiner. Appellants argue adenylate kinase is a ubiquitous protein, which has an activity essential for survival of a host cell or for a viable production process using a host cell, and that adenylate kinase exemplifies polypeptides which hinder the use of polypeptide products according to the presently claimed invention. Appellants' argument is not found persuasive.

It is noted that neither the specification nor the reference of Gilles et al. is sufficient to describe the entire genus of thermolabile adenylate kinases. The specification discloses only two representative species of thermolabile adenylate kinases, i.e., *E. coli* adenylate kinase with mutation at amino acid 87 or 107 and Gilles discloses only a single representative species, i.e., *E. coli* adenylate kinase with mutation at amino acid 87. The recited genus of thermolabile adenylate kinases of claims 81 and 96 encompass species having widely variant structures including any adenylate kinase from any source having any mutation(s) that results in thermolability at 37 degrees Celsius. As such, the two representative species as disclosed in the specification fail to adequately describe the recited genus.

Addressing the group of claims 82 and 97, appellants argue the specification describes *E. coli* adenylate kinase with mutations at positions 87 or 107 as acknowledged by the examiner. Appellants argue adenylate kinase is a ubiquitous protein which has an activity essential for survival of a host cell or for a viable production process using a host cell and that adenylate kinase will likely hinder the use of a polypeptide product. Appellants' argument is not found persuasive.

It is noted that, while the adenylate kinase of claims 82 and 97 may be adequately described, it is noted that the structures of the genus of recited luciferases is NOT adequately described as the genus encompasses species that are widely variant in structure, including luciferase from *any* organism – including those yet to be isolated.

Addressing the group of claim 106, appellants argue the claim is limited to luciferase as the polypeptide product, adenylate kinase that is denatured at temperature of 37 degrees Celsius as the undesired product. Appellants argue each of these aspects of the invention of claim 106 is described in the present specification and are well known to skilled artisans at the time of the invention. Appellants' argument is not found persuasive.

As stated above, and relevant to the instant argument, it is noted that neither the specification nor the reference of Gilles et al. is sufficient to describe the entire genus of thermolabile adenylate kinases recited in claim 106. The specification discloses only two representative species of thermolabile adenylate kinases, i.e., *E. coli* adenylate kinase with mutation at amino acids 87 or 107 and Gilles discloses only a single representative species, i.e., *E. coli* adenylate kinase with mutation at amino acid 87. These two representative species fail to adequately describe the recited genus. Moreover, it is noted that the structures of the genus of recited luciferases is not adequately described as the genus encompasses species that are widely variant in structure, including luciferase from *any* organism including any number of unknown mutations to increase thermostability sufficiently and further including those luciferases yet to be isolated. Thus, the disclosed references referring to specific thermostable luciferase mutants (page 8, lines 20-21 of the specification) fail to represent the entire genus of recited luciferases.

Scope of Enablement Rejection Under 35 USC § 112, First Paragraph

Appellants' arguments addressing the instant rejection begin at page 20 of the Appeal Brief. At the middle of page 20 of the Brief, appellants state the examiner's characterization of the claims in a previous Office action is not understood and request clarification. Appellants argue that, based on the examiner's past statements, claims 81-82, 96-97, and 106 are enabled. Appellants' arguments are not found persuasive.

In the examiner's statement to which appellants refer (i.e., page 6, lines 6-12 of the Office action of Paper No. 30), the examiner set forth that which is enabled by the specification. While the examiner may have provided a statement that differed in scope from that stated above, upon reconsideration of this statement and in view of the enablement provided by the instant specification, the examiner maintains that the specification is enabling only for the scope of the claims which was set forth in the scope of enablement rejection above.

Appellants argue that the specification and the advanced skill in the art at the time of the invention teach one how to make and use the products and methods of claims 72, 80-82, 95-102, and 106. Appellants argue adenylate kinase and luciferase sequences and methods of making and/or screening for temperature sensitive mutants of adenylate kinase and luciferase were known in the art at the time of the invention and recombinant technology was available to make and use cells as claimed. Appellants argue that while some experimentation may be required to make and use the full scope of the claims, such experimentation would not have been undue and to limit the scope of the invention as suggested by the examiner would be "strained and unduly harsh". Appellants argue the cited references describe thermostable luciferases and thermolabile adenylate kinases and either describe methods or methods were well-known for making and screening same. Appellants' arguments are not found persuasive.

It should be noted that claims 98 and 100-102 are not limited to adenylate kinase and luciferase as asserted by appellant and therefore, should not be included in this group. Regarding those claims that specifically recite adenylate kinase and luciferase, it is noted that the claims are not so limited to those thermostable luciferases/thermolabile adenylate kinases that are known in the prior art. With the exception of claims 82 and 97, the claims are not so limited to the thermolabile adenylate kinases referred to in the specification at page 5, lines 1-13 ("[I]t has been found that mutation at position 87 [of *E. coli* adenylate kinase] in that sequence and/or position 107 in the sequence, produces a mutant form of adenylate kinase enzyme which is labile at low temperatures") or those described in the prior art of record. Instead, the claims encompass a broad scope of luciferases and adenylate kinases from any source (including those yet to be isolated) having any mutation(s). It should also be noted that claims 72,

80-82, and 95-102 do not limit the conditions wherein the luciferase remains unaffected and the adenylate kinase is denatured to temperature conditions. Instead, the claims are so broad as to encompass pH conditions as well. There is no evidence of record to indicate or even suggest that the luciferases and adenylate kinases as referred to in the specification and described in the prior art can be used to practice the invention under conditions of altered pH. Not even a single luciferase/adenylate kinase pair that differs in stability to pH or other conditions beyond temperature has been identified in the specification or prior art that would be applicable to practicing the full scope of the claims. As such, the guidance and working examples of thermostable luciferases and adenylate kinases as referred to in the specification and disclosed in the prior art fail to enable the entire scope of claimed methods and products, in particular the broad scope of recited luciferases and adenylate kinases.

Beginning at the top of page 23 of the Brief, appellants argue that, with regard to the remaining claims, the examiner's obviousness rejection of claims 67-78 and 80-106 is inconsistent with the examiner's assertion of a lack of enabling disclosure to enable the entire scope of the claims. Appellants argue that, if the examiner's assertions are correct, *i.e.,* that one of ordinary skill in the art would have had a reasonable expectation of success for making the invention as asserted by the examiner (see page 23, top of the Brief), then the present specification in view of the state of the art, must be sufficient to teach a skilled artisan how to make and use the claimed inventions. Appellants' arguments are not found persuasive.

Contrary to appellants' assertions, the scope of enablement rejection of the claims under 35 USC § 112, first paragraph, is FULLY consistent with the rejection under 35 USC § 103(a). The prior art references cited in the rejection under 35 USC 103(a), while enabling the invention as stated in the rejection, do not combine to enable the entire scope of the claimed invention, as asserted by appellants. In this case, the references cited in the rejection under 35 USC 103(a) combine to enable only a *specific and narrow* scope of the broadly claimed invention, which is fully consistent with the scope of the claims indicated as enabled by the specification. The rejection under 103(a) states that a specific embodiment of the claimed invention is obvious and enabled by the prior art. The rejection under 35 USC 112, first paragraph states that this same embodiment IS enabled but that the full scope of what is claimed is not.

In this case, the specification and the prior art do not teach the entire scope of the invention, particularly for those reasons set forth in the Factors of *In re Wands* described in detail above.

Beginning at the bottom of page 23 of the Brief, appellants argue the examiner's assertion of unpredictability is not supported by any technical or scientific evidence and is not realistic. Appellants argue that no more than routine screening is required to make and use the claimed invention. Appellants argue random mutagenesis of genes of wild-type firefly luciferase as described in Kajiyama et al. (US Patent 5,229,285) is a "viable option" for producing the recited mutant luciferases. Appellants argue similar means may be used to produce mutant adenylate kinases. Appellants argue they should not be required to provide knowledge of the relation of protein structure to function to satisfy the enablement requirement. At the middle of page 24 of the Brief, appellants argue the examiner's conclusion that the combined use of mutant adenylate kinases and mutant luciferases would require an undue amount of experimentation is unsupported and inappropriate. Appellants' arguments are not found persuasive.

Appellants continue to argue limitations that are not present in a majority of the claims. With the exception of claims 72, 80-82, 84, 95-97, 99, and 106, the claims are not so limited to luciferase and adenylate kinase. Instead, the remaining claims encompass any protein product or undesired protein. Furthermore, even those claims limited to luciferase and adenylate kinase (72, 80-82, 84, 95-97, 99, and 106), it is noted that, with the exception of claims 95-97, 99, and 106, the conditions at which the luciferase remains active or stable and the adenylate kinase is denatured are unlimited, i.e., any conditions can be used to denature the adenylate kinase. Even for those claims where the conditions for denaturing adenylate kinase are recited (claims 95-97, 99, and 106), it is noted that the claims are not so limited to mutant luciferases or adenylate kinases from wild-type luciferases or adenylate kinases whose structures are known in the art, *i.e.*, the claims broadly encompass mutants of luciferase and adenylate kinase of wild type luciferases and adenylate kinases yet to be isolated. Appellants' arguments address the routine experimentation of "wild-type firefly luciferase", i.e., *Luciola cruciata*. The examiner acknowledges random mutagenesis as a method that can successfully generate a thermostable luciferase, as was demonstrated by Kajiyama et al. (*Biochemistry* 32:13795-13799) to produce their mutant. However, Kajiyama et al. (*Biochemistry* 32:13795-13799) used a known luciferase sequence as a

template for random mutagenesis. In this case, the claims encompass mutants of luciferases that have yet to be isolated. The claims are not so limited to mutants of known luciferase and adenylate kinase sequences and instead broadly encompass mutants of luciferase (and adenylate kinase) of wild type luciferases (and adenylate kinases) yet to be isolated. As such, the specification and prior art clearly do not provide enablement for making and using the full scope of the claimed invention. Furthermore, for the remainder of the claims that are not limited to luciferase and adenylate kinase, a great deal of additional experimentation might be required to apply random mutagenesis techniques as suitable genes for both the polypeptide product/desired protein and undesired protein might not be available and may not be obtainable. For example, appellants teach that proteases are often undesired proteins. However, identifying the particular protease to mutate may be impossible as most cells often encode numerous proteases. Also, for many polypeptide product/desired protein and undesired protein pairs, there must be suitable conditions under which they differ enough in stability such that the invention will be applicable. Such conditions may not be known and determining them would require undue experimentation.

Beginning at the bottom of page 24 of the Brief, appellants argue the examiner's assertion addressing the undue experimentation required to isolate all mutants of a protein product and an undesired protein as encompassed by the claims is irrelevant. Appellants argue that once a desired and an undesired protein are identified, only routine screening methods are required to make and use the full scope of the claimed invention. Appellants argue they should not be required to teach all such peptides and should not be limited to the disclosed mutants where only routine experimentation may be required to make and use the full scope of the invention. Appellants' arguments are not found persuasive.

It is the examiner's position that the single working example provided in the specification fails to provide the guidance necessary for making and using the claimed invention. It is noted that appellants provide additional examples of where the invention may be usefully applied (pages 13-14 of the Brief). However, until now, appellants have failed to present such evidence. Even assuming *arguendo* such examples had been provided earlier, these examples fail to provide the guidance necessary in making and using the full scope of the invention. In order that one may seek to identify mutants of any protein by recombinant methods, it is first necessary to identify the protein encoding sequence. In the instant case,

the claims are not so limited to those protein, luciferase, or adenylate kinase encoding sequences that are known in the art. Furthermore, as stated above, even those claims limited to luciferase and adenylate kinase (72, 80-82, 84, 95-97, 99, and 106), with the exception of claims 95-97, 99, and 106, the conditions at which the luciferase remains active or stable and the adenylate kinase is denatured are unlimited, i.e., any conditions can be used to denature the adenylate kinase. In essence, appellants' specification would require that a skilled artisan first identify a desired/undesired protein combination within the scope of the claims; isolate all desired/undesired protein encoding sequences; and screen those encoded proteins for the ability of the desired protein to remain active or stable wherein the undesired protein is inactive or denatured under any and all conditions. As such, the specification clearly does not enable the full scope of the claimed invention.

Separate Patentability

Appellants' arguments addressing the separate patentability of the claims rejected for scope of enablement begin at the bottom of page 25 of the Brief. Appellants argue the following groups of claims are separately patentable: claims 67-71; claims 72, 80, 84, 95 and 98-102; claims 81 and 96; claims 82 and 97; claims 73-79, 83, 85-94, and 103-105; and claim 106 are submitted to be each separately patentable one to the other.

Addressing the group of claims 67-71, appellants argue one could identify the mutant forms of the polypeptide product and undesired protein without undue experimentation. Appellants argue the art of record describes the advanced skill in the art for identifying temperature and/or pH sensitive mutants, and the use of recombinant methods to such mutants and means for using same. Appellants argue the use of the combination of proteins identified according to the claims would not have required an undue amount of experimentation. Appellants' argument is not found persuasive.

It is noted that all claims of this group are not so limited to temperature or pH conditions. Instead, the conditions whereby the polypeptide product remains unaffected and the undesired protein is denatured/inactivated are unlimited. As previously stated, while recombinant and mutagenesis techniques are known in the art, it is not routine in the art to screen for all polypeptide products, desired polypeptides,

and undesired polypeptides with an increased or decreased tolerance to any conditions or conditions of temperature or pH as encompassed by the claims. In this case, undue experimentation would clearly be required to practice the entire scope of the claimed invention.

Addressing the group of claims 72, 80, 84, 95 and 98-102, appellants argue the production and use of the recited luciferase and adenylate kinase proteins would not require undue experimentation as the art is replete with examples of the routine nature to make and use these proteins. Appellants' argument is not found persuasive.

It should be noted that, while claims 72, 80, 84, 95 and 99 specifically recite luciferase and adenylate kinase, claims 98 and 100-102 do not recite these limitations as asserted by appellants. Addressing those claims that do recite luciferase and adenylate kinase, it is noted that the claims are not so limited to those examples provided in the prior art and instead broadly encompass luciferases and adenylate kinases from any source – including those yet to be isolated. Furthermore, it is noted that, for example, claim 72 does not limit the conditions under which the luciferase remains unaffected and the adenylate kinase is denatured.

Addressing the group of claims 81 and 96, appellants argue the claims recite adenylate kinase which is thermolabile at a temperature of 37 degrees Celsius, is obtainable from the art without undue experimentation and can be used without undue experimentation. Appellants' argument is not found persuasive.

While the specification and the prior art teach the working examples of a mutant *E. coli* adenylate kinase with mutation at position 87 and 107, it is noted that the claims are not limited to a particular source of the adenylate kinase and/or particular mutations that would provide thermolability to the adenylate kinase. These working examples fail to provide the necessary guidance for making and using the broad scope of the claims.

Addressing the group of claims 82 and 97, appellants argue the examiner acknowledges the *E. coli* adenylate kinase with mutations at positions 87 or 107 is taught in the instant specification. Appellants argue that undue experimentation would not be required to make and use the full scope of the claimed invention. Appellants' argument is not found persuasive.

It is noted that, while the scope of recited adenylate kinases in claims 82 and 97 may be limited to a mutant *E. coli* adenylate kinase with mutation at position 87 and 107, it is noted that the recited luciferase is not so limited to those examples provided in the prior art and instead broadly encompass luciferases from any source – including those yet to be isolated.

Addressing the group of claim 106, appellants argue the use of luciferase as the polypeptide product and adenylate kinase that is denatured at a temperature of 37 degrees Celsius as the undesired product are supported by the specification and generally advanced level of skill in the art.

As stated above, and relevant to the instant argument, it is noted that neither the specification nor the reference of Gilles et al. is sufficient to enable the entire scope of recited adenylate kinases. While the specification and the prior art teach the working examples of a mutant *E. coli* adenylate kinase with mutation at position 87 and 107, it is noted that the claims are not limited to a particular source of the adenylate kinase and/or particular mutations that would provide thermolability to the adenylate kinase. These working examples fail to provide the necessary guidance for making and using the broad scope of the claims. Moreover, it is noted that the entire scope of recited luciferases as encompassed by the claims is not enabled as the scope of recited luciferases encompasses any thermostable luciferase and is not limited to any particular source of luciferase or mutation(s). In this case, the disclosed references referring to specific thermostable luciferase mutants (page 8, lines 20-21 of the specification) fail to enable the entire genus of recited luciferases.

Obviousness Rejection Under 35 USC § 103(a)

Appellants' arguments addressing the instant rejection begin at page 27 of the Brief. At the top of page 28 of the Brief, appellants argue the cited references have been combined through an inappropriate use of hindsight.

In response to appellants' argument that the examiner's conclusion of obviousness is based upon improper hindsight reasoning, it must be recognized that any judgment on obviousness is in a sense necessarily a reconstruction based upon hindsight reasoning. But so long as it takes into account only knowledge which was within the level of ordinary skill at the time the claimed invention was made, and

does not include knowledge gleaned only from the appellants' disclosure, such a reconstruction is proper. See *In re McLaughlin*, 443 F.2d 1392, 170 USPQ 209 (CCPA 1971). As acknowledged by appellants, the thermostable mutant luciferase of Kajiyama et al. (*Biochemistry* 32:13795-13799) and the thermolabile mutant of Gilles et al. were well known at the time of the invention (see page 15, top and page 17, top of the Brief). Furthermore, methods of inactivating contaminating proteins by expressing a thermostable protein in a host and exposing the cell or extract thereof to heat were well known in the art at the time of the invention as evidenced by Backman et al. Thus, teachings of the cited references take into account only knowledge within the level of an ordinarily skilled artisan at the time of the invention and does not include knowledge gleaned from appellants' disclosure.

Addressing the reference of Backman et al. (beginning at the middle of page 28 of the Brief), appellants argue Backman et al. does not teach luciferase production and none of the cited references teaches a luciferase that could withstand the temperatures of the method of Backman et al. Appellants' arguments are not found persuasive.

In response to appellants' arguments against the references individually, it is noted that one cannot show nonobviousness by attacking references individually where the rejections are based on combinations of references. See *In re Keller*, 642 F.2d 413, 208 USPQ 871 (CCPA 1981); *In re Merck & Co.*, 800 F.2d 1091, 231 USPQ 375 (Fed. Cir. 1986). In this case, appellants' arguments fail to recognize the teachings as set forth in Kajiyama et al. (*Biochemistry* 32:13795-13799), Gilles et al., and Belinga et al. It is the combination of these references that renders the claimed invention obvious.

However, in order to fully respond to appellants' arguments, it is noted that Backman et al. present the following method: "a method for obtaining a thermostable enzyme essentially free from unwanted contaminants, characterized in comprising the steps of: (a) providing a mesophilic host cell engineered to express a gene encoding a heterologous thermostable enzyme; (b) culturing said mesophilic host cell to produce said thermostable enzyme in a mixture comprising unwanted contaminants; and (c) purifying said thermostable enzyme said purification comprising at least one step in which a mixture comprising said unwanted contaminants is heated to a temperature sufficient to inactivate said unwanted contaminants but not sufficient to inactivate said thermostable enzyme" (column

2, lines 22-37). To apply the teachings of Backman et al. to the purification of luciferase, one of ordinary skill in the art, based on the teachings of Kajiyama et al. (*Biochemistry* 32:13795-13799) would not apply an extreme temperature of 95 degrees Celsius – Kajiyama et al. (*Biochemistry* 32:13795-13799) clearly teach away from such action as such a temperature would inactivate the luciferase and the instant rejection does not suggest doing so. The rejection states that the selection of appropriate temperature would be obvious – such temperature would clearly be less than 50 degrees Celsius based on the teachings of Kajiyama et al. The luciferase of Kajiyama et al. (*Biochemistry* 32:13795-13799) remains active at 50 degrees Celsius (see page 13796 of Kajiyama et al. *Biochemistry* 32:13795-13799), which is a temperature at which the adenylate kinase of Gilles et al. is inactivated (see page 5798, left column, bottom). Based on the teachings of Backman et al., an ordinarily skilled artisan would have used the minimal temperature sufficient to inactivate the undesired protein(s) (i.e., in the case of luciferase, specifically adenylate kinase). The method presented by Backman et al. (see above) in no way requires a particular or specific temperature for its successful application, thus one of ordinary skill in the art, in view of the teachings of Kajiyama et al. (*Biochemistry* 32:13795-13799), would not have applied temperatures that would inactivate the thermostable luciferase.

Beginning at the bottom of page 28 of the Brief, appellants argue the process of Backman et al. does not require production or engineering of proteins as required by the claimed invention. Appellants argue that a desired protein, *e.g.*, thermostable luciferase produced according to the method of Backman et al. would not require the simultaneous production of an undesired protein that hinders the use of the protein, has an activity essential for cell survival, and has decreased tolerance to a condition compared to the wild-type. In other words, appellants argue the method of Backman et al. provides a process that does not require the identification or use of a mutant undesired protein. Appellants argue that combination of Gilles et al. and/or Belinga et al., which describe adenylate kinase, with Backman et al. would not be logical. to one desiring to produce luciferase according to Backman et al. Appellants' arguments are not found persuasive.

As stated above, the instant rejection is based on a combination of references – not a single or subcombination of the cited references. However, in order to fully respond to appellants' arguments, it is

noted that the examiner acknowledges that the method of Backman et al. does not require generation of a mutant undesired protein as encompassed by the claims. This is precisely what distinguishes Backman et al. from the instant invention (see rejection set forth above). However, no "production or engineering of proteins" is required to practice the method of Backman et al. for inactivating thermolabile adenylate kinase in the presence of a thermostable luciferase. One of ordinary skill in the art would have recognized that production of thermostable luciferase using a mesophilic bacterial host such as *E. coli* according to the method of Backman et al. would not ensure removal of endogenously expressed wild-type *E. coli* adenylate kinase because the lucifease is not stable at a sufficiently high enough temperature to remove all of the adenylate kinase. Thus, in order to ensure removal of residual adenylate kinase activity, one would have used a mesophilic host cell expressing a thermolabile adenylate kinase, which was known in the art at the time of the invention as evidenced by Gilles et al. This is clearly suggested by Backman et al., who teach, "[m]esophilic host cells are cells which can be engineered to produce the desired thermophilic enzyme and whose proteins generally are denatured at a temperature that does not denature the desired thermophilic enzyme" (column 2, lines 45-49). One merely need transform the *E. coli* bacterium of Gilles et al., expressing the thermolabile mutant adenylate kinase, with the vector of Kajiyama et al. (*Biochemistry* 32:13795-13799), expressing a thermostable luciferase, to practice the method of Backman et al. to remove adenylate kinase activity. Thus, contrary to appellants' assertions, no "production or engineering of proteins" is required to practice the method of Backman et al. and in view of the teachings of Belinga et al. describing the presence of adenylate kinase as an interfering contaminant in preparations of luciferase, it would have been "logical" to combine the cited references.

Beginning at the bottom of page 29 of the Brief, appellants argue that in reading Kajiyama et al. (*Biochemistry* 32:13795-13799) and Backman et al., one would at best be motivated to produce luciferases that would be sufficiently thermostable to be expressed in the mesophilic host of Backman et al. that would withstand the temperatures according to Backman et al. of 80-95 degrees Celsius. Appellants argue the thermostable luciferase of Kajiayama et al. (*Biochemistry* 32:13795-13799) was only tested at 50 degrees Celsius. Appellants argue that, even if in reading Kajiyama et al. (*Biochemistry* 32:13795-13799) and Backman et al., one were motivated to produce luciferase mutants with even

greater thermostability, this is not the subject of the invention. Appellants' assert that luciferases are thermolabile enzymes. Appellants present the reference of Wood et al. (WO 99/14336) as supporting the unrealistic expectation of obtaining a luciferase mutant that is able to withstand temperatures for use in the method of Backman et al. Appellants argue the thermostable enzymes of Backman et al. are derived from bacteria isolated from hot springs and Backman et al. rely on the thermostability of these enzymes to operate their method. Appellants argue luciferase is derived from organisms that exist in ambient temperatures and achieving thermostability equivalent to the enzyme of Backman et al. is unreasonable. Appellants' arguments are not found persuasive.

It should be noted that the examiner can find no previous citation of the reference of Wood et al. However, as this reference presents no new issues and is similar to the references of, *e.g.,* Lowe et al., Kajiyama et al. (*Biochemistry* 32:13795-13799) and Kajiyama et al. (US Patent 5,229,285), the reference has been considered by the examiner. It should also be noted that the instant rejection is based on a combination of references – not a single cited reference or a subcombination of the cited references as argued by appellants. However, in order to fully respond to appellants' arguments, it is noted that appellants' arguments fail to recognize the teachings of Gilles et al. and Belinga et al., which combined with the teachings of Kajiyama et al. (*Biochemistry* 32:13795-13799) and Backman et al., render the claimed invention obvious. As stated above, the method of Backman et al. (see above) in no way requires a particular or specific temperature for its successful application, thus one of ordinary skill in the art, in view of the teachings of Kajiyama et al. (*Biochemistry* 32:13795-13799), would not have applied temperatures that would inactivate their thermostable luciferase. In view of the teachings of Kajiyama et al. (*Biochemistry* 32:13795-13799) one of ordinary skill in the art would not apply extremes of temperature of 80-95 degrees Celsius in order to inactivate adenylate kinase – Kajiyama et al. (*Biochemistry* 32:13795-13799) clearly teach away from such action as such temperatures would inactivate their luciferase. The luciferase of Kajiyama et al. (*Biochemistry* 32:13795-13799) remains active at 50 degrees Celsius (see page 13796 of Kajiyama et al. *Biochemistry* 32:13795-13799), which is a temperature at which the adenylate kinase of Gilles et al. is inactivated (see page 5798, left column, bottom). Thus, an ordinarily skilled artisan would have used the minimal temperature necessary to inactivate the

temperature sensitive mutant *E. coli* adenylate kinase of Gilles et al. – not temperatures of 80-95 degrees Celsius as asserted by appellants.

At page 31 of the Brief, appellants argue that one reading Gilles et al. and Backman et al., with or without Kajiyama et al. (*Biochemistry* 32:13795-13799), would have been interested in producing adenylate kinase according to Backman et al. and would have been "frustrated" by the decrease in thermostability of the adenylate kinase of Gilles et al. as Backman et al. uses thermostable enzymes in their method. Appellants argue that the combined references of Gilles et al. and Backman et al. would not have taught how to make thermostable enzymes for use in the method of Backman et al. Appellants argue that the claimed invention requires thermolabile adenylate kinase whereas Backman et al. suggests production of thermostable enzymes at temperatures of 85-90 degrees Celsius. Appellants argue that it is unclear why one of ordinary skill in the art would combine Gilles et al. and Backman et al., with or without the references of Kajiyama et al. (*Biochemistry* 32:13795-13799) and Belinga et al. to make the claimed invention. Appellants' arguments are not found persuasive.

As stated above, the instant rejection is based on a combination of references – not a single or subcombination of the cited references. However, in order to fully respond to appellants' arguments, it is noted that appellants argue the references of Gilles et al. and Backman et al. combine to teach a method of creating thermostable adenylate kinases according to the method of Backman et al. This is clearly not the case. Gilles et al. characterize their adenylate kinase as a "[t]hermosensitive adenylate kinase of *E. coli*" (page 5798, left column, bottom). The method of Backman et al. is used in the production of "thermostable" proteins. Thus, one of ordinary skill in the art would not attempt such a futile exercise for attempting to create thermostable mutants of an enzyme that is itself already rendered thermosensitive by mutation, i.e., why would one attempt to create a thermostable enzyme of a mutant whose corresponding wild-type is more thermostable? In this case, appellants have failed to recognize the additional teachings of the other cited references. The rejection is based on a combination of four references – not a subcombination thereof, which combine to render obvious the claimed invention.

Beginning at the bottom of page 31 of the Brief, appellants argue the examiner's interpretation of Belinga et al. is contrary to Backman et al., who describe expression of a single heterologous gene

encoding a thermostable enzyme. Appellants argue that one would have read from the combination of references that a more thermostable luciferase as compared to that of Kajiyama et al. (*Biochemistry* 32:13795-13799) is required for the method of Backman et al. Appellants argue the combination of cited references would teach that heating and purification by the method of Backman et al. would eliminate adenylate kinase, but would also eliminate luciferase. Appellants note the method of Belinga et al. does not require the use of luciferase or adenylate kinase mutants or recombinant methods. Appellants argue that because Belinga et al. teach a method of purifying luciferase to 95% total enzyme activity that one would not have been motivated to provide further methods for purifying luciferase from adenylate kinase. Appellants' arguments are not found persuasive.

As stated above, the instant rejection is based on a combination of references – not a single or subcombination of the cited references. However, in order to fully respond to appellants' arguments, it is noted that, as stated above, the method of Backman et al. (see above) in no way requires a particular or specific temperature for its successful application, thus one of ordinary skill in the art, in view of the teachings of Kajiyama et al. (*Biochemistry* 32:13795-13799), would not have applied temperatures that would inactivate their thermostable luciferase. In view of the teachings of Kajiyama et al. (*Biochemistry* 32:13795-13799) one of ordinary skill in the art would not apply extremes of temperature of 80-95 degrees Celsius in order to inactivate adenylate kinase – Kajiyama et al. (*Biochemistry* 32:13795-13799) clearly teach away from such action as such temperatures would inactivate their luciferase. The luciferase of Kajiyama et al. (*Biochemistry* 32:13795-13799) remains active at 50 degrees Celsius (see page 13796 of Kajiyama et al. *Biochemistry* 32:13795-13799), which is a temperature at which the adenylate kinase of Gilles et al. is inactivated (see page 5798, left column, bottom). Thus, an ordinarily skilled artisan would have used the minimal temperature necessary to inactivate the temperature sensitive mutant *E. coli* adenylate kinase of Gilles et al. – not temperatures of 80-95 degrees Celsius as asserted by appellants. Furthermore, while the method of Belinga et al. is not directed to producing mutant enzymes by recombinant techniques, the reference clearly provides motivation for removing adenylate kinase from a luciferase preparation. The knowledge of an expression vector encoding a thermostable luciferase mutant and an *E. coli* host expressing a thermolabile adenylate kinase are provided by Kajiyama et al.

(*Biochemistry* 32:13795-13799) and Gilles et al., i.e., it is the combination of ALL cited references – not a single reference or subcombination of references – that render obvious the claimed invention.

Separate Patentability

Appellants' arguments addressing the separate patentability of the claims rejected as being obvious to an ordinarily skilled artisan at the time of the invention begin at the bottom of page 32 of the Brief. Appellants argue the following groups of claims are separately patentable: claims 67-71; claims 72, 80, 84, 95 and 98-102; claims 81 and 96; claims 82 and 97; claims 73-78, 83, 85-94, and 103-105; and claim 106 are submitted to be each separately patentable one to the other.

Addressing the group of claims 67-71, appellants argue the cited art fails to teach or suggest the combined identification of the mutant forms of the polypeptide product and undesired protein as required by the claims. Applicants argue the art of record, such as Kajiyama et al. (*Biochemistry* 32:13795-13799) discloses only the identification of a thermostable luciferase and not the combination of two proteins and Backman et al. teaches the use of a thermostable protein. Appellants argue the combination of proteins would not have been obvious from the combination of cited art. Appellants' argument is not found persuasive.

Appellants continue to argue against the cited references individually or in subcombination. In this case, appellants' arguments address only the references of Kajiyama et al. (*Biochemistry* 32:13795-13799) and Backman et al. and have failed to recognize the teachings of the remaining references. One cannot show nonobviousness by attacking references individually where the rejections are based on combinations of references. See *In re Keller*, 642 F.2d 413, 208 USPQ 871 (CCPA 1981); *In re Merck & Co.*, 800 F.2d 1091, 231 USPQ 375 (Fed. Cir. 1986). In this case, the rejection is based on a combination of four references – not a subcombination thereof.

Addressing the group of claims 72, 80, 84, 95 and 98-102, appellants argue Belinga et al. teach a method of complete purification of luciferase such that the claimed method is a further, patentably distinct method of producing luciferase free of adenylate kinase. Appellants argue the post-production purification method of Belinga et al. and/or production of a single thermostable luciferase according to Backman et al.

would not have made the invention obvious as the claims require production of mutated forms of luciferase and adenylate kinase. Appellants' argument is not found persuasive.

It is noted that the method of Belinga et al. is not recited in the instant claims such that this method can be distinguished as being a "further, patentably distinct method" as asserted by appellant. In this case, appellants address the references of Belinga et al. and Backman et al. without considering the teachings of the remaining cited references. Again, it is noted that the rejection is based on a combination of four references – not a subcombination thereof.

Addressing the group of claims 81 and 96, appellants argue the limitation of an adenylate kinase which is thermosensitive at a temperature of 37 degrees Celsius is not suggested by the prior art because Backman et al. teach the production of proteins which are thermostable at temperatures of 85 degrees Celsius, which teaches away from the claimed invention. Appellants' argument is not found persuasive.

Appellants address the reference of Backman et al. without considering the teachings of the remaining cited references. Again, it is noted that the rejection is based on a combination of four references – not a subcombination thereof. Regarding the limitation of an adenylate kinase which is thermosensitive at a temperature of 37 degrees Celsius, it is noted that the reference of Gilles et al. clearly suggests the mutant adenylate kinase has increased susceptibility to denaturation and proteolysis at 37 degrees Celsius compared to the wild-type enzyme.

Addressing the group of claims 82 and 97, appellants argue the inclusion of the limitation of an *E. coli* adenylate kinase with mutation at position 87 or 107 with luciferase would not have been obvious from the cited art. Appellants' argument is not found persuasive.

The further limitation of the *E. coli* adenylate kinase with mutation at position 87 or 107 is clearly taught by Gilles et al. and, in combination with the other cited references, renders the claimed invention obvious to one of ordinary skill in the art.

Addressing the group of claim 106, appellants argue the claim recites luciferase that is thermostable at a temperature of 37 degrees Celsius and adenylate kinase that is denatured at a

temperature of 37 degrees Celsius, which are preferred combinations of the elements which exemplify the claimed invention. Appellants' argument is not found persuasive.

It is noted that, while claim 106 may be a preferred embodiment of the claimed invention, the combination of cited references makes obvious ALL of claims 67-78 and 80-106. The examiner has addressed all limitations of the claims and submits that claim 106 is no more separately patentable than any of the other rejected claims under 35 USC 103(a).

For the above reasons, it is believed that the rejections should be sustained.

Respectfully submitted,

David J. Steadman
November 17, 2003

Conferees

Ponnathapura Achutamurthy
Supervisory Patent Examiner
Art Unit 1651

Michael Wityshyn
Supervisory Patent Examiner
Art Unit 1651

Michael G. Wityshyn (CONFEREE)
Supervisory Patent Examiner
Technology Center 1600

Rebecca E. Prouty
Primary Examiner
Art Unit 1652

NIXON & VANDERHYE, PC
1100 N GLEBE ROAD
8TH FLOOR
ARLINGTON, VA 22201-4714